

The Impact of Adherence to a Vegan Diet on Acid-Base Balance:  
A Randomized Controlled Trial in Healthy College Students

by

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## ABSTRACT

There is a considerable amount of research stating that vegetarian diets have an alkalizing effect while the typical western diet is acid-forming. There is substantial evidence regarding the health benefits of an alkaline diet. Although vegetarian diets demonstrate the ability to foster these health benefits, many people are still not willing to adopt a completely vegetarian diet. PURPOSE: To evaluate the effect of following a vegan diet two or three days per week on acid-base balance in a healthy college student population aged 18-30. METHODS: In a one-week interventional design, 23 people were randomly assigned to follow a vegan diet 2 days per week (VEG2;n=7), 3 days per week(VEG3;n=8), or 7 days per week (VEG7;n=8). Urine pH and dietary PRAL were assessed in each group at baseline and after the one-week intervention. RESULTS: There was no significant difference in urinary pH between the three groups ( $p=0.12$ ). The change in PRAL values after the dietary intervention was different between the 3 groups ( $p=0.03$ ). CONCLUSION: Adherence to a vegan diet 2 or 3 days per week did not show a significant change in urinary pH or PRAL.

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## CHAPTER 1

### INTRODUCTION

Vegetarian diets are gaining popularity in the United States and other developed countries. According to a 2008 survey, 7.3 million Americans follow a vegetarian diet (Vegetarian Times, 2008). There are many variations of the vegetarian diet. Two common variations are strict vegetarians (vegans) and lacto-ovo vegetarians. Vegans eliminate all products derived from animals from their diet, and lacto-ovo vegetarians consume eggs and dairy while eliminating all flesh foods (Craig, 2010). There are many reasons for the increased interest in adopting vegetarian diets. According to a qualitative study conducted in 2007, personal health and opposition to animal cruelty were among the most popular reasons for consuming a vegetarian diet (Fox & Ward, 2008). Other motivating factors included religious purposes, environmental concerns, and social influences (Fox & Ward, 2008).

The health benefits of vegetarian diets have recently become apparent as more research has been, and continues to be, conducted on the topic. Many of these health benefits can be attributed to the composition of well-planned vegetarian diets. Generally, vegetarian diets are low in cholesterol and saturated fat and high in fiber and phytochemicals (Craig, 2010). Vegetarian diets are also usually richer in fruits and vegetables, which can give vegetarian diets an alkalizing effect when compared to omnivorous diets (Deriemaeker, Aerenhouts, Hebbelinck, & Clarys, 2010).

This alkalizing effect is important because there must be a balance between acids and alkali in the body in order to carry out functions efficiently. There is a plethora of evidence to substantiate the effect that food intake has on acid-base balance in the human

body (Deriemaeker et al., 2010). Generally, protein has an acidifying effect in the body while fruits and vegetables have an alkalizing effect (Deriemaeker et al., 2010). Because of high protein and low fruit and vegetable consumption, the standard diet in today's industrialized society is acid-forming, which may play a role in the increased prevalence of chronic diseases (Scialla & Anderson, 2013).

Acid-base balance within the human body is a complexly regulated system. Acid is produced in the body when sulfur, found in the amino acids cysteine and methionine, is metabolized to form sulfuric acid (Scialla & Anderson, 2013). Bicarbonate is an alkaline product that is produced from organic anion salts found in high amounts in fruits and vegetables (Scialla & Anderson, 2013). The difference between these acidic and basic products of metabolism yields the dietary acid load (Scialla & Anderson, 2013).

One measurement used to estimate dietary acid load is called the potential renal acid load, or PRAL. PRAL allows for the estimation of how much net acidity certain foods will form within the body (Alexy, Kersting, & Remer, 2008). PRAL allows for the estimation of dietary acid load through the use of dietary intake data alone (Alexy et al., 2008). The most commonly used measures of dietary acid load are PRAL or 24-hour urine (Scialla & Anderson, 2013). The 24 hour urine collection allows for the testing of net acid excretion (NAE) and pH. (Scialla & Anderson, 2013). The urinary markers are the most direct measurements of dietary acid load (Scialla & Anderson, 2013).

Acid-base balance is a concern, because an imbalance can cause physiological problems which can lead to chronic disease (Scialla & Anderson, 2013). Metabolic acidosis can result in bone and muscle loss as well as kidney disease (Scialla & Anderson, 2013). Although it has been demonstrated that vegetarian diets reduce the acid

load in the body, as well as many other health benefits, the majority of the population is not willing to make such a drastic lifestyle change. No research has been published that describes how often one must follow a vegan diet to see an effect on acid-base balance within the body. If consuming a vegan diet only two or three days per week demonstrated an alkalizing effect, it would be a much more feasible goal for the general population.

This study will compare the dietary acid load, as determined by PRAL and urine pH, of subjects following a vegan diet 7 days per week (VEG7), 3 days (VEG3) per week, and 2 days per week (VEG2).

### **Purpose**

The purpose of this randomized controlled trial is to evaluate the effect of following a vegan diet two to seven days per week on acid-base balance in healthy college students between 18 and 30 years old.

### **Null Hypotheses**

- $H_{01}$  = There will be no difference in urinary pH between the group consuming a plant-based diet seven days per week and the group consuming a plant-based diet two days per week.
- $H_{02}$  = There will be no difference in urinary pH between the group consuming a plant-based diet seven days per week and the intervention group consuming a plant-based diet three days per week.
- $H_{03}$  = There will be no change in urinary pH of the VEG7 group between the baseline and post-intervention measurements.

## **Operational Definitions**

For the present study, the following operational definitions applied:

1. PRAL referred to potential renal acid load or the net amount of acid produced by the metabolism of a given food.
2. Vegan referred to a diet that includes no animal derived ingredients.
3. NAE referred to net acid excretion – a urinary marker of acid produced in the body.

## **Delimitations**

The following delimitation applies to this study:

1. Subjects were students at the College of Health Solutions at Arizona State University.
2. Subjects were healthy adults ranging from 18-30 years old.
3. Subjects were not taking prescribed medication other than oral contraceptives.
4. Subjects were non-smokers.
5. Subjects were not pregnant or lactating.
6. Subjects were not competitive athletes.

## **Limitations**

The following limitations applied to this study:

1. Subjects' honesty when self-reporting on their dietary logs

2. Non-adherence to dietary intervention
3. Incomplete 24-hr urine collections

## CHAPTER 2

### REVIEW OF LITERATURE

The purpose of this randomized controlled trial was to determine the effect of consuming a vegan diet 2, 3, and 7-days out of a week on acid-base balance in a healthy college student population. This chapter includes a review of literature that is relevant to the topics of this study, including mechanisms of regulation of acid-base balance, dietary influence on acid-base balance, other factors influencing acid-base balance, assessment of dietary acid load, health implications of acid-base balance, health implications of vegetarian diets, and the relationship between vegetarian diets and acid-base balance, and dietary patterns of the Greek Orthodox population.

#### **Regulation of Acid-Base Balance**

Acid-base homeostasis within the body involves many processes. Maintenance of acid-base balance requires the intestines, liver, lungs, kidneys, and buffering systems. The pH of the blood is tightly regulated to fall within the range of 7.35-7.45. When the blood pH falls above or below the normal range, alkalosis or acidosis results, respectively (Ayers & Dixon, 2012).

The main function of the lungs in acid-base homeostasis is the regulation of the partial pressure of carbon dioxide and oxygenation of the blood (Ayers & Dixon, 2012). The intestines are involved directly in acid and base production. The liver plays a major role in the regulation of acid-base balance as well. In the liver, large amounts of hydrogen and alkali ions are produced by oxidizing sulfur-containing amino acids and organic ions, respectively. At first, these ions are buffered through the use of intracellular fluid buffers. After being released into circulation, they add to the acid-base pool in the blood. The

blood is buffered not only by extracellular fluid buffers, but also through pulmonary mechanisms. The lungs can alter the rate of carbon dioxide excretion in proportion to the alteration in blood bicarbonate levels. This is called respiratory compensation and allows the blood pH to be maintained within a narrow range. Although the lungs are able to regulate pH by changing the partial pressure of carbon dioxide, this process does not allow for the loss or gain of hydrogen ions. The lungs are also not able to regenerate lost bicarbonate. The kidneys are the final regulators in the system of acid-base balance. They are able to produce bicarbonate and eliminate an amount of acid that is equal to endogenously produced acid to maintain a balance between acid and base (Remer, 2000).

The kidneys play an essential function in maintaining acid-base balance by maintaining bicarbonate concentration in the blood, forming titratable acid, and excreting ammonium in the urine. The kidneys are mainly responsible for moderating dietary influence of acid-base balance (Ayers & Dixon, 2012).

Metabolic acidosis occurs when the acid produced through metabolism surpasses the neutralizing base-forming compounds produced. Being that the kidneys play a main role in this aspect of acid-base homeostasis and kidney function tends to decline with age, metabolic acidosis becomes more of a concern in older populations. As kidney function declines, the body cannot neutralize the acids formed during metabolism as efficiently or to as great of an extent as when kidneys are functioning at full capacity (Adeva & Souto, 2011).

Acid-base balance can also be regulated through altering gene expression. A study published in 1991 assessed the effect of acid-base balance on renal glutaminase and phosphoenolpyruvate carboxykinase gene expression in rats. Acidosis was induced by

administering 1.5%  $\text{NH}_4\text{Cl}$  as a drinking water source. The acidosis that resulted caused a 6-fold increase in the levels of glutaminase and phosphoenolpyruvate carboxykinase mRNA. These are key regulatory enzymes of renal ammoniogenesis, which is a method of neutralizing endogenous acid. In regular acid-base balance, metabolism produces equivalent amounts of bicarbonate and ammonium ions which produce urea. During an acidotic state, urea synthesis is decreased and there is an increase in the hepatic synthesis of glutamine. As the synthesis of glutamine in the liver is increased, an increase in glutamine catabolism in the kidneys occurs. This glutamine catabolism results in an increase of ammonium ions in the urine which allows for the excretion of acid without the use of sodium and potassium ions. Also, the breakdown of glutamine to glucose creates bicarbonate which can partially compensate for the systemic acidosis (Hwang & Curthoys, 1991).

### **Dietary Influence on Acid-Base Balance**

The concept of diet playing a major role in acid-base homeostasis within the body is not a new one. An article published in 1968, described in detail the mechanism in which sulfur-containing amino acids, methionine and cysteine, are metabolized to produce acid within the body. These amino acids found in high amounts in animal protein are metabolized to yield sulfuric acid. Researchers were trying to determine which components of food had an effect on acid and base production during metabolism. They fed subjects various elemental formulas that were so basic that they were not supposed to have any effect on endogenous acid production. Then they tried to work on an algorithm to determine what components of a whole food diet would need to be accounted for when estimating endogenous acid production. At this point, acid-base balance was not as well



understood, so the researchers were unable to determine which specific nutrients would need to be accounted for in a formula to estimate dietary acid load (Lennon & Lemann, 1968).

Frassetto et al. published an article in 1998 introducing a method of estimating net acid excretion (NAE) in humans using dietary potassium and protein contents. He conducted a study in which 42 subjects between the ages of 17 and 73 were recruited and consumed one of six whole foods diets while residing in the University of California, San Francisco General Clinical Research Center. Subjects consumed their diets for  $\geq 1$  week, which is a period that has been shown in previous literature to be an adequate amount of time to establish steady-state acid-base equilibrium (Kurtz, Maher, Hulter, Schambelan, & Sebastian, 1983). Diets were analyzed using direct chemical analysis or diet-composition tables. NAE was estimated using the following formula with values obtained from dietary intake:

$$\text{Estimated NAE} = 0.94 \text{ protein} - 0.61 \text{ potassium} + 22.$$

A correlation was observed between the ratio of dietary protein to potassium calculated using the formula above and NAE obtained from 24-hour urine samples ( $r = 0.84$ ,  $p < 0.001$ ). This formula developed by Frassetto et al is still one of the methods used to estimate NAE (Frassetto, Todd, Morris, & Sebastian, 1998).

The main dietary factors that influence the body's acid load are sulfur from the catabolism of cysteine and methionine, phosphorus, potassium, magnesium, and calcium. Sulfur and phosphorus are the acid-forming nutrients while potassium, magnesium, and calcium are the base-forming nutrients. Potential renal acid load (PRAL) is a formula that

uses the amounts of these nutrients in the diet to estimate the acid load of the diet. PRAL is calculated using the following formula:

$$\begin{aligned}\text{PRAL (mEq/d)} = & 0.49 \times \text{protein (g/d)} \\ & +0.037 \times \text{phosphorous (mg/d)} \\ & -0.021 \times \text{potassium (mg/d)} \\ & -0.026 \times \text{magnesium (mg/d)} \\ & -0.013 \times \text{calcium (mg/d)}\end{aligned}$$

A negative PRAL value indicates a more alkalizing food whereas a positive value indicates an acid-producing food (Remer, Dimitriou, & Manz, 2003).

A study conducted in children and adolescents between the ages of 3 and 18 explored the impact of food groups, age, and time trends on PRAL. The study population demonstrated an acidic diet as evident by a positive PRAL value. Researchers discovered that fruits had the most alkalizing effect on the subjects' diets due to the quantity in which they were consumed. Vegetables did not produce as significant of an alkalizing effect simply due to the fact that they were not consumed as much. The meat/fish/eggs category of food yielded the highest acidifying effect. PRAL was significantly higher in males when compared to females across all ages that were included in this study ( $p < 0.0001$ ) (Alexy et al., 2008).

The term net endogenous acid production (NEAP) is used to describe the diet-dependent acid that is produced within the body during metabolism. NEAP can be estimated by calculating renal NAE using the following formula:

$$\text{Renal NAE (mEq/day)} = [\text{Titrateable acid}] + [\text{ammonium}] - [\text{bicarbonate}]$$

These values can be measured using a 24-hour urine sample (Adeva & Souto, 2011).

In one of the first studies that examined a dietary intervention's ability to directly affect acid-base balance and urinary pH, Remer et al. recruited 6 subjects to consume 3 different diet types: low-protein, moderate-protein, and high-protein. The study utilized a repeated measures design where all subjects consumed all 3 diets in the same order. Each diet was consumed for 5 days at a time. During the final 2 days of each diet period, 24-hour urine samples were collected. Renal NAE measurements and urinary pH demonstrated a significant difference between the acid-forming effects of each of the 3 diets. Using a two-way ANOVA for repeated measures, researchers discovered that the different diets had statistically significant effects on urine pH and NAE as well as urinary excretion of phosphate, sulfate, potassium, and calcium ( $p < 0.001$ ) (Remer & Manz, 1994).

Results of this study showed that as protein in the diet increased, so too did urinary acidity. The urinary pH level that was reached while subjects followed the high protein diet was very close to the urinary pH that marks maximum stimulation of renal acid excretion. When urinary pH falls below 5.4, it signifies that the kidneys have reached their capacity for getting rid of acid loads within the body. When subjects followed the high protein diet, the mean urinary pH was  $5.5 \pm 0.2$ . As the kidneys' capacity for ridding the body of acid is surpassed, metabolic acidosis results (Remer & Manz, 1994).

Diet has such a profound and consistent effect on acid-base balance within the body that numerous formulas have been developed to calculate estimated NAE based on dietary intake alone. One such formula was analyzed for its validity in a study conducted

by Remer et al. The formula estimated net acid excretion using dietary intakes of chloride, phosphorus, sulfate, sodium, potassium, calcium, and magnesium. The formula accounted for intestinal net absorption of each specific electrolyte included in the formula. This formula estimates NAE by finding the difference of the sum of the nonbicarbonate ions minus the sum of the mineral cations. The formula is as follows:

$$\text{NAE indirect} = (\text{chloride} + \text{phosphorus} + \text{sulfate} + \text{organic acids}) - (\text{sodium} + \text{potassium} + \text{calcium} + \text{magnesium}).$$

All of these values are based on levels found in the urine. It was shown that the formula accurately estimated NAE (Remer, 2000).

In an article published in 2001, Frassetto et al explored the diet composition of human's ancestors in relation to acid-base balance. Before the agricultural revolution and industrialization, humans consumed much greater amounts of potassium than the current American diet provides. The current American diet has replaced the potassium-rich plant foods that used to be consumed in abundance with processed foods containing high levels of sodium chloride. It is estimated that in ancestral hunter-gatherer societies, sodium intake was about 29 mEq day and potassium intake exceeded 280 mEq per day. The contemporary human diet contains an estimated 80 mEq of potassium per day and between 100 and 300 mEq of sodium per day. Now sodium levels are surpassing the levels of acid-neutralizing potassium. This disturbance in acid-base balance is thought to have a deleterious effect on health and could possibly be implicated in the increased incidence of chronic disease since the industrialization of our food system (Frassetto, Morris, Sellmeyer, & Sebastian, 2008).

In a study published in 2006 that was conducted to determine the effect of grains on acid-base balance, subjects were randomized into one of two groups: meat plus fruits and vegetables or meat plus cereal grains for a 74-day trial. By the end of the trial, the NAE of the fruit and vegetable group was  $44.7 \pm 21.6$  mmol/d compared to  $56.0 \pm 23$  in the cereals group ( $p < 0.001$ ). This study demonstrates the acidifying effect of cereal grains compared to the alkalizing effect of fruits and vegetables (Jajoo, Song, Rasmussen, Harris, & Dawson-Hughes, 2006).

A study published in 2008 demonstrated a plant based supplement's ability to increase urinary pH. Urinary pH has been shown to be a reliable estimator of dietary acid load. In this study, 34 men and women measured and recorded the pH of a spot sample of their urine for 7 days to establish a baseline measurement. Subjects then continued to measure their urine pH for 14 more days while consuming the plant-based supplement. There was a significant increase in urinary pH ( $p = 0.03$ ) (Berardi, Logan, & Rao, 2008). This study exemplifies the ability of dietary intake to significantly influence urinary pH even in a short time frame of 2 weeks.

The literature has shown that in general, an increased consumption of fruits and vegetables decreases the body's acid load by contributing base-forming electrolytes. A study published by Welch et al in 2008 used plasma vitamin C as a biomarker to indicate fruit and vegetable consumption. A positive relationship was found between plasma vitamin C status and urine pH measured from 24-hour urine samples with a p value of  $< 0.001$  in both male and female subjects. Researchers also discovered a significant continuous relationship between plasma vitamin C levels and urine pH after adjusting for possible covariates. Researchers looked at mean plasma vitamin C levels stratified by

units of pH. Subjects were grouped by their urinary pH and the average plasma vitamin C was calculated for each group. The p values for the trends found in both men and women were <0.001 (Welch, Mulligan, Bingham, & Khaw, 2008).

A 2009 study of healthy Hong Kong adolescents assessed the estimated net endogenous acid production in relation to dietary intake of bone health-related nutrients. Investigators used the algorithm developed by Frassetto et al to estimate net endogenous acid production. This algorithm assesses the diet's protein to potassium ratio. Researchers found that net endogenous acid production of the Hong Kong adolescents increased with the consumption of meat and was reduced with intake of fruits, vegetables, and legumes (Chan, Woo, Chan, Cheung, & Lo, 2009).

### **Other Factors Influencing Acid-Base Balance**

While diet plays a crucial role in acid-base balance within the body, it is important to understand other factors that can have an influence on acid-base homeostasis. One main factor that determines acid-bases status is the kidneys' ability to neutralize or rid the body of acid. A paper published by Ayer et al discussed the factors affecting renal regulation of acid-base balance. They determined three factors to be most important: the rate at which the body can excrete acid-base buffers, the strength of the buffers, and the extent to which plasma bicarbonate is reduced (Ayer, Schiess, & Pitts, 1947).

A study published in 2000 by Henger et al. examined the effects of the inhibition of aldosterone action by spironolactone and of angiotensin II action by losartan on regulation of acid-base homeostasis. For this study, 8 subjects with pre-existing metabolic acidosis were recruited. Throughout the three study periods, a constant

metabolic diet was consumed by the subjects. In the first phase of the study,  $\text{NH}_4\text{Cl}$  was administered to induce an increased acidotic state. Once the acidosis was considered to be in a steady state, the subjects moved on to the next phase of the study. For this study, subjects were considered to be in a steady state when plasma bicarbonate concentration varied by no more than 1.5 mmol/L and  $\text{P}_{\text{CO}_2}$  varied by no more than 3 mm Hg. In the next phase of the study, oral spironolactone was administered in addition to the  $\text{NH}_4\text{Cl}$  until the acidosis was considered to be in a steady state once again. Finally, in the third phase of the study, losartan was administered in addition to the  $\text{NH}_4\text{Cl}$  and spironolactone until a steady state of acidosis was achieved once again. The administration of spironolactone caused a significant increase in NAE when compared to the administration of solely  $\text{NH}_4\text{Cl}$  ( $p < 0.005$ ). Urinary sulfate excretion also increased significantly ( $p < 0.05$ ). The worsening of the metabolic acidosis was found to be caused by extra-renal effects. When the losartan was administered as well, a worsening of the metabolic acidotic state was found and was attributed to renal effects (Henger, Tutt, Riesen, Hultner, & Krapf, 2000).

Because both spironolactone and losartan act as angiotensin II antagonists, this study demonstrated the interaction between angiotensin II and the regulation of acid-base balance. When angiotensin II receptors are blocked, such as when spironolactone or losartan are administered, metabolic acidosis is worsened. This study proposed that angiotensin II agonism is an important modulator of distal nephron acidification and can, in turn, modulate the severity of metabolic acidosis (Henger et al., 2000).

### **Assessment of Dietary Acid Load**

Urine pH measured from a 24-hour urine sample has been found to be an accurate measure of dietary acid load. A study published in 2007 by Welch et al using data from the EPIC-Norfolk population study as well as additional data derived from a subset of the population used in the EPIC-Norfolk study examined relationships between dietary intake and urinary pH. Researchers used both seven day food diaries and a validated food frequency questionnaire to calculate PRAL. Casual urine samples and 24-hour urine samples were used to measure urinary pH. Researchers discovered the strongest relationship between 24-hour urine pH and the dietary information derived from the seven day food diary. Welch et al found a relationship between dietary PRAL and urine pH using the aforementioned measurements. The  $\beta$  coefficient of the relationship between PRAL and urine pH was -0.08 units of pH for each standard deviation of PRAL. This relationship was significant at  $p < 0.001$  (Welch et al., 2008).

Another study investigated the efficacy of using PRAL to estimate net acid excretion in children and adolescents. For this study, a total of 238 healthy children and adolescents were recruited to participate. They completed a diet record and collected a 24-hour urine sample. A significant relationship was found between NAE and PRAL in both the children ( $r = 0.43$ ) and adolescents ( $r = 0.51$ ). These relationships were significant with a p value of  $<0.001$ . The study concluded that PRAL was effective at estimating net acid excretion in child and adolescent populations (Remer et al., 2003).

### **Health Implications of Acid-Base Balance**

Acid-base balance has been shown to affect several different systems and processes within the body. Chronic metabolic acidosis occurs when excess acid is



generated in the body or when the acid produced in the body cannot be removed entirely through normal homeostatic mechanisms. Both aging and excessive meat consumption have been implicated in the development of metabolic acidosis (Alpern & Sakhaee, 1997). In many cases, blood pH and serum  $\text{HCO}_3^-$  levels may appear normal even in chronic metabolic acidosis because the body has many homeostatic mechanisms to maintain these levels in a very narrow range. The compensatory mechanisms that are used by the body during chronic metabolic acidosis can have deleterious effects on the body including nephrolithiasis, bone demineralization, muscle degradation, and renal growth (Alpern & Sakhaee, 1997). The main acid-base-related health effects that have been investigated in the literature are: bone resorption, hypertension, muscle wasting, chronic low-back pain, kidney disease, hormonal changes, and changes in athletic performance.

### **Acid-Base Balance and Bone Health**

The majority of research conducted on acid-base balance and its impact on health has been done in terms of bone health. There is evidence that the Western diet increases the risk of osteoporosis by increasing acid levels within the body. Bone acts as a buffering system for acid-base balance within the body. Neutralizing cations such as calcium can be released from the bone matrix in order to maintain systemic pH in a normal range. This can be problematic because the Western diet is generally acid-forming. This becomes more of a concern in older populations as kidney function declines with age which reduces the body's ability to eliminate excess endogenous acid. This increases the likelihood and severity of metabolic acidosis which can cause

problems for bone health since bone resorption can occur in order to release ions to neutralize the systemic acidity (Wynn, Krieg, Lanham-New, & Burckhardt, 2010).

A study published in 1992 aimed to measure the effects of chronic metabolic acidosis on calcium, phosphate, and 1,25-(OH)<sub>2</sub>D metabolism in addition to serum parathyroid hormone levels. Eight healthy, male subjects were recruited to participate in the study. At the start of the study, the subjects consumed a constant metabolic diet without NH<sub>4</sub>Cl supplementation. This portion of the study served as a control. After being studied for 4 days following the control diet, subjects were randomly divided into two groups. One group of four subjects continued following the constant metabolic diet with an additional supplementation of 2.1 mmol NH<sub>4</sub>Cl/kg of body weight. The remaining four subjects also continued with the constant metabolic diet but were supplemented with 4.2 mmol NH<sub>4</sub>Cl/kg body weight. Subjects were observed for eight days on their respective NH<sub>4</sub>Cl supplementation interventions. During this time, a new steady state of acid-base balance was established in all of the subjects. Subjects were considered to be in a steady state when plasma values of bicarbonate varied by no more than 1.5 mmol/liter and PaCO<sub>2</sub> values varied by less than 3 mmHg for three consecutive days. Blood samples were drawn on steady state days at approximately 6pm to analyze parathyroid hormone, 1,25-(OH)<sub>2</sub>D, calcium, and phosphate. This study showed a significant decrease in parathyroid hormone levels in the high dose NH<sub>4</sub>Cl group of  $4.9 \pm 1.8$  pg/ml ( $p < 0.025$ ). Researchers found that a state of metabolic acidosis increased the production rate as well as the metabolic clearance rate of 1,25-(OH)<sub>2</sub>D. In more severe acidotic states, the increased production rate is greater than the increased metabolic clearance rate, which results in a significant increase of 1,25-(OH)<sub>2</sub>D in the

plasma. This study showed a significant increase in serum 1,25-(OH)<sub>2</sub>D in the high dose NH<sub>4</sub>Cl group of 15.8 ±2.9 pg/ml (p<0.001) (Krapf, Vetsch, Vetsch, & Hulter, 1992).

The Framingham Osteoporosis study, published in 2001, was a cohort study where participants ranged from 69-97 years old at baseline. Bone mineral density was measured two times, four years apart, and dietary intake was assessed using a food frequency questionnaire at baseline. Investigators discovered that increased consumption of base-forming food groups resulted in less bone loss, but contrary to the study's hypothesis, increased protein consumption was also associated with decreased bone loss. It was hypothesized that increased protein in the diet would lead to increased bone loss due to greater acid production within the body. The opposite was found to be true in this cohort study. Researchers postulated that this relationship between protein and bone mineral density exists because protein is an integral and important component of bone structure and so adequate levels of protein are necessary to maintain bone mineral density. The study concluded that adequate levels of fruits and vegetables as well as protein-rich foods are important in maintaining bone mineral density (Tucker, Hannan, & Kiel, 2001).

A paper published in 2003 explored the effect of acid-base balance on the regulation of bone cell function. There are two major cell types that are responsible for bone growth and turnover: osteoblasts and osteoclasts. Osteoblasts play a role in bone mineralization while osteoclasts play a role in the resorption of bone. It has been found that bone resorption by osteoclasts is dependent on extracellular acidification. Osteoclasts show no activity when pH levels are above about 7.3, and they are most active when pH is around 6.9. Generally, cell function is inhibited or impaired in acidic conditions, but

osteoclasts are stimulated when pH levels drop. It is thought that this is a compensatory method that evolved to cause alkalizing minerals to be leached from the bone when pH levels dropped and the lungs and kidney are not able to compensate (Arnett, 2003).

A study conducted on 337 postmenopausal women examined the influence of dietary potassium and NEAP on bone mineral density and markers of bone resorption. Bone mineral density and urinary markers of bone resorption were collected as well as dietary information through the use of a food frequency questionnaire. Subject data was stratified into quartiles based on dietary potassium intake. Researchers compared the highest quartile to the lowest quartile and found a difference of 8% in bone mineral density between the two groups. This study suggests that potassium intake could have an effect on bone mineral density, and thus the risk of developing osteoporosis (Macdonald, New, Fraser, Campbell, & Reid, 2005).

A study conducted at Tufts University on 40 healthy men and women 50 years of age and over, published in 2006, showed that an increase in dietary acid load was associated with markers of bone health including calcium excretion, biochemical markers relating to bone turnover and parathyroid hormone levels. This trial showed a change in NAE was correlated with change in serum PTH ( $r = 0.358$ ,  $p = 0.023$ ). A change in NAE was also significantly associated with a change in calcium excretion in the urine ( $r = 0.381$ ,  $p = 0.020$ ). These correlations suggest a relationship between dietary acid load and bone health and resorption (Jajoo et al., 2006).

In 2009, a study was published that examined the effect of potassium citrate supplementation on bone quality in renal transplant patients. This intervention study was conducted over 12 months, and 30 renal transplant patients with metabolic acidosis

participated. Half of the subjects received a potassium citrate supplement (intervention group) and the other half received a potassium chloride supplement (control group). Bone biopsies of the iliac crest and dual-energy X-ray absorptiometry were performed at baseline and at the end of the 12-month trial. At the end of the trial, bone surface ( $p < 0.01$ ), connectivity density ( $p = 0.01$ ), and cortical porosity ( $p = 0.05$ ) were better preserved with the potassium citrate when compared to the control group receiving potassium chloride. No significant changes were found in bone mineral density between the two groups (Starke et al., 2012).

### **Acid-Base Balance and Blood Pressure**

The first known study to investigate a possible relationship between acid-base balance and blood pressure was published by Murakami et al in 2007. The study was conducted on 1,154 free-living Japanese women between 18 and 22 years of age. Dietary habits were assessed using a self-administered diet history questionnaire. PRAL and the protein to potassium ratio were both used in this study to estimate dietary acid load. Cardiometabolic risk factors were assessed. These included blood pressure, weight, BMI, LDL and HDL-cholesterol and serum TAG concentration. A positive correlation was found between both systolic and diastolic blood pressure and acidic dietary loads. This association was more significant for systolic blood pressure ( $p = 0.028$ ) than for diastolic blood pressure ( $p = 0.035$ ), but both were significant. Investigators also found an independent positive correlation between PRAL and total ( $p = 0.042$ ) and LDL-cholesterols ( $p = 0.021$ ) (Murakami, Sasaki, Takahashi, Uenishi, & Japan Dietetic Students' Study for Nutrition and Biomarkers Group, 2008).

The next study conducted on the relationship between diet-dependent acid-base balance and blood pressure was conducted on a population of women in the United States. In this prospective study, 87,293 women were followed for 14 years. Investigators discovered that an increased diet-dependent acid load was independently correlated with hypertension risk. Dietary acid load was approximated using estimated NEAP. After controlling for age, BMI, and physical activity, NEAP showed a positive correlation with protein intake (correlation coefficient: 0.96;  $p < 0.001$ ) and a negative correlation with potassium intake (correlation coefficient: -0.96;  $p < 0.001$ ). Estimated NEAP was positively correlated with hypertension risk ( $p = 0.003$ ) (Zhang, Curhan, & Forman, 2009).

A more recent study, published in 2012, examined the relationship between dietary acid load and hypertension risk in both men and women. A total of 2241 Dutch adults over the age of 54 were examined. Dietary information and baseline blood pressure was measured. Six years later, the subjects returned and blood pressure was recorded once again to determine rate of hypertension. These data were analyzed in terms of PRAL and NEAP that were calculated using baseline dietary data. Subjects were placed into tertiles based on PRAL and NEAP scores to determine if risk of hypertension differed between the groups. After controlling for age, sex, BMI, smoking, education, and intakes of alcohol, fiber, and total energy, no significant relationship was observed between dietary acid load and hypertension ( $p = 0.83$ ). Investigators did not collect dietary information after the baseline collection, so there is a possibility that subjects' diets had changed within the 6 years of the study. Dietary PRAL and NEAP were calculated using only baseline dietary information. Investigators also postulated that the

Dutch diet might not reach levels that are acidic enough to influence blood pressure (Engberink et al., 2012).

In 2013, a preliminary research study was conducted on the effect of acid-base balance on blood pressure in healthy children and adolescents. A cross-sectional study of 267 healthy children ranging in age from 4-14 years old demonstrated a positive but non-significant correlation between dietary acid load and systolic blood pressure. Subjects completed weighted 3-day food logs and collected 24-hour urine samples. At each annual visit, anthropometric measurements were taken. Blood pressure was also measured at each visit. Although a trend was observed where as PRAL increased, so too did hypertension risk, this relationship was not statistically significant ( $p = 0.06$ ). It is thought that a possible relationship between dietary acid load and hypertension risk could be due to increased cortisol production. Increased cortisol has shown a positive correlation with risk of hypertension. It has also been postulated that impaired insulin-like growth factor I (IGF-I) due to metabolic acidosis could be responsible for the increased hypertension risk (Krupp, Shi, Maser-Gluth, Pietzarka, & Remer, 2013). The relationship between acid-base balance and blood pressure warrants further investigation as a relationship has been determined in several different populations, but is non-conclusive.

### **Acid-Base Balance and Muscle Maintenance**

The maintenance of lean body mass is a relevant health concern as sarcopenia, or reduction in muscle mass and strength, is common in elderly populations. This decrease in muscle mass is attributed to increased incidence of falls and decreased mobility in elderly people. It is thought that increased muscle wasting in elderly people can be

attributed to a decrease in renal function that increases the acidity of the body, which, in turn, breaks down muscle in the body in order to produce neutralizing ammonium (Welch, MacGregor, Skinner, Spector, Moayyeri, & Cassidy, 2013b).

An alkaline status has been shown to preserve lean tissue mass in adults prone to muscle wasting. A study published in 2008 demonstrated that subjects who were 65 years of age or older who consumed a more alkaline diet had a greater percentage of lean body mass than those who consumed a more acid-forming diet. A total of 384 subjects were analyzed who had previously completed the National Institute on Aging Sites Testing Osteoporosis Prevention/Intervention Treatment (STOP/IT) trials at Tufts University. The trial lasted for 3 years, during which subjects were randomly assigned to a treatment group that consumed a supplement with calcium plus vitamin D or a control group with a double placebo. Lean body mass (LBM) was measured using DXA. Researchers discovered that increased excretion of potassium (indicative of increased potassium consumption) was associated with greater LBM% in both men and women ( $p < 0.001$ ) (Dawson-Hughes, Harris, & Ceglia, 2008).

This decrease in LBM% in subjects consuming an acidic diet is thought to be caused by an adaptive process that the body undergoes during acidosis. Muscles are broken down to release amino acids into the bloodstream to allow the liver to produce glutamine which is then used to synthesize ammonia which is a neutralizing agent for acid produced within the body (Dawson-Hughes et al., 2008).

A study conducted by Welch et al. in 2013 examined women ranging from 18-79 years old and demonstrated a greater lean body mass percentage in women with a more alkaline diet across all age ranges. Subjects were recruited from the TwinsUK Registry,



which is an ongoing study of adult twins in the UK. This study includes data from 2689 of the female twins. All subjects completed DXA body scans, dietary questionnaires and clinical assessments between 1996 and 2000. Investigators explored the relationship between body composition and PRAL. After controlling for age, physical activity, smoking, and fat mass, subjects consuming a more alkaline diet had an average fat free mass (FFM) that was 0.79kg higher than subjects consuming an acidic diet ( $p = 0.001$ ). Percentage of fat free mass (FFM%) was also examined. After adjusting for age, physical activity, and smoking, the difference in FFM% between those consuming alkaline diets versus acidic diets was 1.06% ( $p < 0.001$ ) (Welch, MacGregor, Skinner, Spector, Moayyeri, & Cassidy, 2013a).

#### **Acid-Base Balance and Chronic Low Back Pain**

In a clinical trial conducted in 82 subjects with chronic low back pain, subjects received an alkaline multi-mineral supplement for 4 weeks in addition to whatever medications they were already taking. Intracellular mineral content in sublingual cells was measured and venous blood samples were analyzed. After taking the supplement, the buffering capacity of blood was significantly increased from  $77.69 \pm 6.79$  to  $80.16 \pm 5.24$  mmol/L ( $p < 0.001$ ). Investigators used the Arhus low back pain rating scale (ARS) to quantify the low-back pain of the subjects. The ARS pain scale measured pain intensity, disability, and physical impairment. Researchers discovered a significant reduction in low-back pain in subjects after taking the alkaline-forming supplement for 4 weeks ( $p = 0.001$ ). The effect of acid-base status on chronic low back pain requires further investigation as this is the only known study to investigate this relationship. Chronic low back pain is a common ailment in the United States, and its relationship with acid-base

balance could have clinical relevance (Vormann, Worlitschek, Goedecke, & Silver, 2001).

### **Acid –Base Balance and Kidney Disease**

The kidneys play a vital role in maintaining acid-base balance within the body. Net endogenous acid that is produced within the body must be excreted or neutralized by the kidneys to maintain acid-base balance (Scialla & Anderson, 2013).

In a study of 632 African Americans diagnosed with hypertensive chronic kidney disease, a diet resulting in increased endogenous acid production was correlated with a faster rate of chronic kidney disease progression. This study suggested that protein intake was not as influential on disease progression as was the ratio between protein and natural sources of alkali, such as fruits and vegetables. This means that chronic kidney disease might not progress more quickly due to higher protein intakes as long as the higher protein intakes are counterbalanced by increased fruit and vegetable consumption to neutralize the acid formed by protein metabolism (Scialla et al., 2012).

A recent study of the treatment of metabolic acidosis in stage 4 hypertensive kidney disease investigated the efficacy of using fruits and vegetables or sodium bicarbonate as a method of treatment. The study was conducted on 111 patients with stage 4 hypertensive kidney disease. Subjects were randomly assigned to either 1 year of daily oral  $\text{NaHCO}_3$  or an intervention consisting of increased fruit and vegetable consumption. Investigators measured estimated glomerular filtration rate (eGFR) to assess kidney function as well as urine measures of kidney injury. After one year of intervention, the group receiving fruits and vegetables did not differ from the group receiving  $\text{NaHCO}_3$  in terms of eGFR. The fruits and vegetables group had lower-than-

baseline measurement of urinary components indicative of kidney injury. The 8-hour NAE differed significantly in both the fruits and vegetables group and the  $\text{NaHCO}_3$  group when compared to their baseline measures ( $p < 0.01$ ). The results from this study demonstrated that a dietary intervention increasing consumption of fruits and vegetables can improve metabolic acidosis and reduce further injury of the kidneys in stage 4 hypertensive kidney disease (Goraya, Simoni, Jo, & Wesson, 2013).

Urine pH also mitigates the risk of developing uric acid kidney stones. When urine pH is above the  $\text{pK}_a$  value of uric acid (5.51), an increased amount of uric acid is found in its base form of urate. Urate is relatively soluble and does not cause kidney stone formation. As urine pH falls below 5.51, however, the insoluble uric acid increases in predominance and precipitates to form kidney stones. It has been found that urine pH is one of the main pathogenic findings in uric acid stone formers (Burns & Finlayson, 1984).

### **Acid-Base Balance and Hormonal Changes**

A study published in 1998 by Remer et al examined the short-term impact of a vegetarian diet on adrenal androgens and adrenocortical activity. For this study, a repeated measures design was used to assess the effects of three different diets in six healthy adults. The three diets that were administered were a normal diet, a protein-rich diet, and a lactovegetarian diet. Each diet was followed for 5 days. First the subjects followed the normal diet, followed by the protein rich diet, followed by the lactovegetarian diet, followed by the normal diet once again. All food was prepared for the subjects and was measured and weighed. 24-hour urine samples were collected at the end of each diet period as well as venous blood samples. Remer et al. found a significant

decrease in urinary 24-hour excretion rates of dehydroepiandrosterone sulfate ( $p<0.01$ ), total 17-ketosteroid sulfates ( $p<0.005$ ), cortisol ( $p<0.01$ ), and creatinine ( $p<0.001$ ) when the subjects followed the lactovegetarian diet as opposed to the normal diet. The article concluded that a low protein vegetarian diet can reduce adrenocortical activity. Because a low protein vegetarian diet has been demonstrated to have an alkalizing effect, the effects of a vegetarian diet on adrenocortical activity might be attributed to changes in acid-base balance (Remer, Pietrzik, & Manz, 1998).

In 2004, a paper was published by McCarty which summarized data leading to a hypothesis that acid-base balance plays a role in insulin resistance syndrome by modulating cortisol output (McCarty, 2005). A crossover study conducted by Remer that studied the effect of a normal protein omnivore diet, a high protein omnivore diet, and a low protein vegetarian diet discovered that 24 hour urinary excretion of cortisol was about 30% lower in the vegetarian group than in the normal protein group ( $p<0.01$ ). Cortisol excretion was higher in the high protein group when compared to the normal protein group, although the difference was not statistically significant (Remer, 2001).

In a cross-sectional study conducted by Longscope et al, dietary information and serum concentrations of sex hormone-binding globulin were assessed in 1709 men between the ages of 40 and 70. Dietary information was obtained using the Willett semiquantitative 1-year food frequency questionnaire. This study found a negative correlation between SHBG and dietary protein intake ( $p=0.03$ ) (Longscope, Feldman, McKinlay, & Araujo, 2000). Remer suggested that this decrease in SHBG in the presence of a high protein diet is due to the fact that cortisol is a negative modulator of SHBG.

Since high protein diets could lead to an increase in cortisol production, the increase in cortisol could then decrease levels of SHBG (Remer, 2001).

Maurer et al. conducted a study in which serum cortisol and urinary excretion of cortisol metabolites were measured in subjects while they were consuming sodium and potassium chloride, and then later when the sodium and potassium chloride were replaced with sodium and potassium bicarbonate. All other factors of the diet remained consistent throughout the study period. When the subjects were consuming the bicarbonate, plasma cortisol, urinary cortisol and cortisol metabolite levels were significantly lower ( $p < 0.05$ ) (Maurer, Riesen, Muser, Hulter, & Krapf, 2003).

In 2007, a study was conducted to examine the relationship between urinary pH and metabolic syndrome. Metabolic syndrome is characterized by several different metabolic factors including dyslipidemia, hyperglycemia, hypertension, obesity, and insulin resistance (Eckel, Grundy, & Zimmet, 2005). In this cross-sectional study, 148 participants collected 24 hour urine samples, gave a fasting blood sample, and had their height, weight, and blood pressure measured. Investigators determined whether each subject had metabolic syndrome or not using the definition of metabolic syndrome from the American Heart Association and the National Heart, Lung, and Blood Institute (Grundy et al., 2005). Subjects with metabolic syndrome demonstrated a significantly lower urine pH when compared to subjects without metabolic syndrome ( $p < 0.001$ ). Mean urine pH was also examined in terms of the number of metabolic syndrome features. Mean urine pH decreased as number of metabolic syndrome features increased ( $p < 0.0001$  for trend). Inverse correlation was also observed between urine pH and degree of insulin resistance ( $p < 0.0001$ ). This inverse correlation remained significant after

controlling for certain factors such as age, gender, BMI, creatinine clearance, and urine sulfate. The mechanism behind the inverse correlation between degree of insulin resistance and urine pH is unclear. This area requires further research (Maalouf, Cameron, Moe, Adams-Huet, & Sakhaee, 2007).

### **Acid-Base Balance and Athletic Performance**

An area of increased interest in the acid-base balance research community has been how acid-base balance might affect athletic performance. Hydrogen ions accumulate in muscles as they work which is thought to be one factor that causes fatigue (Williams, 1995). Ergogenic aids are increasing in popularity in competitive sports, so if simple dietary changes or even sodium bicarbonate supplementation can show improvements in athletic performance by reducing or delaying muscle fatigue, it could be a feasible and economical tool for athletes to use in their training.

A study conducted in 2010 on boxing athletes examined the effect of sodium bicarbonate supplementation on boxing performance. Ten subjects were recruited for this study. Subjects were randomized into two groups. One group received 0.3g/kg body weight of sodium bicarbonate while the other group received a placebo. Then the boxers were paired up for 4 sparring bouts. Each round lasted 3 minutes with 1 minute between each round for recovery. Blood acid-base, base excess, performance, and total punches landed were recorded. There was a significant correlation between administration of sodium bicarbonate and base excess ( $p < 0.001$ ). The subjects who received the sodium bicarbonate had increased blood buffering capacity before and after the sparring rounds. The sodium bicarbonate group also demonstrated a significant increase in punches landed when compared to the placebo group ( $p < 0.001$ ) (Siegler & Hirscher, 2010).

Another study, published in 2012, examined the effects of sodium bicarbonate supplementation on lower-body resistance exercise. Twelve participants were recruited for this randomized counterbalanced trial. Participants ingested either 0.3g/kg body weight of sodium bicarbonate or a placebo 60 minutes prior to the resistance exercise trial. The resistance protocol consisted of several lower body exercises. Performance in these exercises were determined based on total repetitions during each exercise, total repetitions throughout the trial, and a performance test consisting of a knee extension exercise. Researchers found that sodium bicarbonate administration resulted in significantly more total repetitions when compared to the placebo ( $p < 0.05$ ) as well as significantly greater levels of blood lactate after the exercise trial ( $p < 0.05$ ). The study showed an increase in repetitions in the sodium bicarbonate group as exercise volume accumulated. There was no significant difference in performance in the first two exercises ( $p = 0.18$  and  $p = 0.06$ , respectively). This is consistent with the idea that sodium bicarbonate allows for a greater buffering potential when the muscles begin to accumulate hydrogen ions, thus delaying the onset of fatigue (Carr, Webster, Boyd, Hudson, & Scheett, 2013).

### **Relationship between Vegetarian Diets and Acid-Base Balance**

Vegetarian diets have been shown to decrease dietary acid load. This is thought to be because high protein intake has an acid forming effect while high intake of fruits and vegetables has an alkalizing effect. Vegetarian diets are generally higher in fruits and vegetables and lower in protein (specifically sulfur-containing amino acids found in high amounts in animal protein that results in increased dietary acid load) than omnivorous diets.

A study published in 1997 explored the relationship between vegetarian diets and blood and urine acid-base status in premenopausal women. Thirty-three women were recruited for the study, 20 were omnivores and the remaining 13 followed a vegetarian diet (abstaining from flesh foods, but consuming dairy and eggs). Subjects recorded their food and beverage intake for 7 consecutive days and gave blood and urine samples. Although calcium intake did not differ significantly between the two groups, calcium excretion was significantly higher in the omnivores ( $p = 0.014$ ). A significant difference was found between the two groups in respect to urinary titratable acid. Mean urinary titratable acid output in the omnivores was  $48.9 \pm 20.3$  mEq/24h while mean urinary titratable acid in the vegetarian group was  $35.3 \pm 23.3$  mEq/24h ( $p = 0.014$ ). The mean urine pH in the omnivore group tended to be lower than that of the vegetarian group but was not statistically significant ( $p = 0.17$ ). Mean blood pH was actually higher in the omnivorous group than in the vegetarian group ( $p = 0.05$ ). This is the opposite relationship as would be expected. The study had a small sample size and investigators mentioned that some subjects in the vegetarian group occasionally ate fish. It is possible that differences in blood pH were not seen as expected due to non-compliance or misrepresenting dietary information. All literature published since this article have demonstrated that vegetarian diets are associated with more alkaline outcomes (Ball & Maughan, 1997).

A study published in 2010 analyzed differences in dietary intake between vegetarians and omnivores in terms of dietary acid production. Based on dietary records of 60 subjects (30 vegetarian and 30 omnivorous), researchers determined that the dietary intake of omnivores was more acid forming than dietary intakes of vegetarians based on



calculated dietary PRAL. Mean PRAL values demonstrated an alkaline dietary load for vegetarians with an average PRAL value of  $-5.4 \pm 14.4 \text{ mEq/day}$ . Omnivores demonstrated an acidic dietary load with an average PRAL value of  $10.3 \pm 14.4 \text{ mEq/day}$ . A negative PRAL level indicates an alkaline diet whereas a positive PRAL indicates an acid diet. The difference observed between the PRAL levels of the vegetarian and non-vegetarian groups was statistically significant ( $p < 0.001$ ). The data obtained from this study were consistent with the hypothesis that omnivores are at greater risk of diet-induced metabolic acidosis than their vegetarian counterparts (Deriemaeker et al., 2010).

The study conducted by Welch et al using data from the EPIC-Norfolk study also found a relationship between a vegetarian diet and acid-base balance. In both women and men, urine pH was more alkaline in the subjects who were vegetarian as opposed to omnivorous. Vegetarian men had an average urine pH of 6.1 whereas omnivorous men had an average urine pH of 5.9 ( $p < 0.001$ ). Vegetarian women had an average urine pH of 6.0 and omnivorous women had an average urine pH of 5.9 ( $p = 0.003$ ). This relationship remained significant after adjusting for covariates. It is important to note that the vegetarian subjects in this study only abstained from flesh foods (meat, poultry, fish) and were not strict vegetarian (abstaining from eggs and dairy as well) (Welch et al., 2008).

A study published in 2012 by Hietvala et al examined the effect of a low-protein vegetarian diet on blood acid-base status. In this cross-over study, nine active men were randomly assigned either to the low-protein vegetarian diet group or the normal diet group. Both groups followed their assigned diet for 4 days, followed by a 10-16 day washout period, after which the groups would switch and follow the other diet for four days. Researchers found no difference in venous blood pH between the vegetarian and

normal diet groups (Hietavala, Puurtinen, Kainulainen, & Mero, 2012). It is not surprising that no difference in venous blood pH was seen after such a short-term dietary intervention. Blood pH is tightly regulated using a complex system of buffers, and a change in blood pH would not be expected to be seen in such a short-term intervention (Ayers & Dixon, 2012).

### **Health Implications of Vegetarian Diets**

The health benefits of vegetarian diets have been well documented in the literature. A cohort study examined the mortality rates of vegetarians and omnivores in a Seventh Day Adventist population. A total of 96,469 participants were recruited between 2002 and 2007. The study resulted in overall reduced mortality risk in vegetarians compared to omnivores. Vegetarians were found to have lower mortality rates from specific causes as well. Vegetarian diets were associated with lower cardiovascular mortality, and lower non-cardiovascular non-cancer mortality, which was notably attributed to reduced mortality from diseases with endocrine or renal causes (Orlich et al., 2013).

The GEICO study was a randomized controlled trial that explored the use of a plant-based nutrition intervention to reduce body weight and the risk for cardiovascular disease in a corporate setting. A total of 291 subjects participated in the study. Subjects were selected if they were at least 18 years old, had a BMI of at least 25 and/or had been diagnosed with type 2 diabetes. A total of 10 GEICO corporate offices participated in the study. Each site was randomly assigned to be in the intervention or the control group. All participants in each corporate office were in the same group. The subjects in the intervention group followed a low-fat vegan diet for 18 weeks. There were no calorie

restrictions set. Measurements were taken at week 0 and week 18. Investigators measured body weight, height, blood pressure, plasma cholesterol and triglycerides, and HbA<sub>1c</sub>. When compared to the control group, the intervention group showed significant improvements in total cholesterol ( $p < 0.01$ ), LDL-cholesterol ( $p < 0.001$ ), body weight ( $p < 0.001$ ), and hemoglobin A<sub>1c</sub> ( $p < 0.01$ ). These findings were significant as improvements in these areas could markedly reduce the risk of the development of many chronic diseases that are becoming an increased concern (Mishra et al., 2013).

The EPIC-Oxford cohort study examined the risk of hospitalization and death from ischemic heart disease. A group of 44, 561 men and women were studied, out of whom 34% followed a vegetarian diet. Vegetarians were shown to have a lower BMI, non-HDL cholesterol, and systolic blood pressure than non-vegetarians. The vegetarians in the cohort also had a 32% lower risk of ischemic heart disease than their non-vegetarian counterparts (Crowe, Appleby, Travis, & Key, 2013).

In a cross-sectional study on Seventh Day Adventists, vegetarians were shown to have healthier mood states than omnivores. Generally, it has been shown that high intakes of omega-3 fatty acids are beneficial for mental health and mood states. Vegetarian diets tend to be low in omega-3 fatty acids, but vegetarians still had improved mood states when compared to non-vegetarians. A total of 138 participants were recruited for this cross-sectional study. DASS and POMS scores were used to measure mood states. The average total POMS scores were significantly lower in vegetarian participants than in omnivore participants ( $p = 0.007$ ). Mean DASS scores were also lower in vegetarian participants than omnivore participants ( $p < 0.001$ ). These findings

demonstrate a positive correlation between vegetarian diets and healthy mood states (Beezhold, Johnston, & Daigle, 2010).

A later randomized controlled trial investigated the relationship between vegetarian diets and improved mood states. The results of the randomized controlled trial supported the results of the cross-sectional study, showing that after omnivores eliminated meat and fish from their diets for two weeks, their mood states improved. This improvement in mood states associated with consuming a vegetarian diet could be due to the fact that vegetarian diets are much lower in arachidonic acid which is known to cause inflammation and increase risk for depression. In this trial 39 subjects were recruited and randomly assigned to the omnivore, fish, or vegetarian group. The omnivore group continued following their normal diet, the fish group eliminated all meat but consumed a certain amount of fish, and the vegetarian group eliminated all meat and fish. Subjects followed their prescribed diets for 2 weeks. After 2 weeks, the vegetarian group showed the greatest decrease in DASS-stress scores compared to both the omnivore and fish groups ( $p = 0.045$ ). This decrease in DASS-stress scores from baseline suggests that the vegetarian diet improved mood parameters (Beezhold & Johnston, 2012).

### **Dietary Patterns of the Greek Orthodox Population**

Traditional Greek Orthodox Christians follow a Mediterranean diet pattern that is known for its health benefits. One element of the Greek Orthodox diet that is commonly overlooked when researching diet composition is the intermittent fasting that Greek Orthodox Christians practice. Greek Orthodox Christians undergo fasting almost every Wednesday and Friday as well as for longer periods during 3 major periods during the year. During these fasting periods, practitioners eliminate all animal products, including

flesh foods, dairy, and eggs, from their diets (Papadaki, Vardavas, Hatzis, & Kafatos, 2008). This is significant for this study, because it demonstrates a population that regularly follows the proposed dietary intervention. It is a sustainable practice for this group of people, so it might also be feasible for others. There have also been health benefits associated with this dietary pattern. It is possible that some of these benefits could be correlated with an alkalizing effect of the diet.

In addition to fasting almost every Wednesday and Friday, longer fasting periods occur 3 times per year. These periods include the 40 days before Christmas, 48 days before Easter, and 15 days in August (the Assumption). A study published in 2008 examined the effect of these fasting periods on serum lipids and obesity. Investigators recruited 120 subjects, 60 were considered regular fasters while the other 60 subjects did not practice fasting and acted as the control group. Measurements were obtained from both groups at the beginning and end of each fasting period for a total of 6 measurements in a one-year period. When compared to the control group, the fasting group had significantly decreased levels of total cholesterol, LDL-C, LDL/HDL-C ratio, and BMI (Sarri, Tzanakis, Linardakis, Mamalakis, & Kafatos, 2003).

Although the Greek Orthodox population's diet has been studied for its health benefits, no known study has determined how this dietary pattern of consuming a strict vegetarian diet two days per week might affect acid-base balance. This dietary pattern that could be feasible for the general population to decrease incidence of diet-induced metabolic acidosis.

## CHAPTER 3

### METHODOLOGY

The purpose of this randomized controlled trial was to evaluate the effect of consuming a vegan diet two, three, or seven days per week on acid-base balance in a healthy college student population. This chapter presents the participants, study design, variables, procedures, laboratory analyses, and statistical analyses.

#### **Participants**

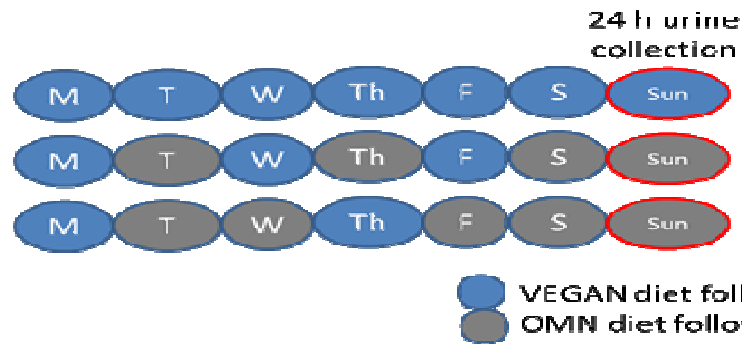
Thirty healthy adult omnivores ranging from 18-30 years old were recruited from the College of Health Solutions at Arizona State University. Subjects were recruited through email list serves within the nutrition department and asked to fill out an online survey to demonstrate potential eligibility. Prospective study participants were excluded if they smoke, have a history of significant medical problems, are on a prescribed medication besides oral contraceptives, are competitive athletes, do not consume meat every day, or are pregnant or lactating.

Approval for this study was obtained from the Arizona State University Internal Review Board (Appendix A). All subjects signed an informed consent form before participating in the study (Appendix B).

#### **Study Design**

In this parallel-arm randomized controlled trial, 30 subjects were randomly assigned to one of three groups. The VEG2 (n=7) group followed a vegan diet for 2 days out of the week, the VEG3 (n=10) group followed a vegan diet for 3 days out of the week, and the VEG7 (n=9) group followed a vegan diet for 7 days out of the week.

Figure 1  
*Vegan Day Distribution for Dietary Intervention Groups*



Differences in acid-base balance between the three groups were assessed using 24-hour urine pH and dietary potential renal acid load (PRAL) calculated using a 7-day food diary.

### Variables

The independent variable in this experimental trial is the number of days the subjects follow a vegan diet. VEG2 consumed a vegan diet 2 days of the week, VEG3 consumed a vegan diet 3 days out of the week and VEG7 consumed a vegan diet all 7 days of the week.

The dependent variables are urine pH measured from 24-hour urine samples and PRAL calculated from the 7-day food diaries. These variables will be assessed in order to determine whether or not there is a difference in acid-base balance between the three groups.

### Procedures

Visit 1 took place on a weekday. At this visit, subjects filled out a consent form (Appendix B) and a medical history questionnaire (Appendix C) as well as getting their height, weight, and blood pressure measured. Subjects were then given a vegan-eating tutorial as well as a helpful handout to assist them in making vegan food choices

(Appendix D). At this visit, the subjects were also given the materials and instructions they need to complete their 3-day food diaries (Appendix E), 7-day food diaries, and two 24-hour urine collections.

Subjects filled out the 3-day food log from Thursday through Saturday while consuming their regular diet. On the following Sunday, subjects collected their first 24-hour urine collection which they brought into the lab the following day (Monday), which constituted the second visit. On that same Monday, subjects began their 7-day dietary intervention and began filling out their 7-day food diary, recording all of their food and beverage intake for each of the 7 days of the dietary intervention. Reminders were sent to participants to ensure that they followed a vegan diet on the days which had been assigned to them as well as to ensure that they are not having problems making vegan food choices. On the final day of the dietary intervention (Sunday), subjects collected their second 24-hour urine sample. Subjects came in to the lab on the following Monday for their third, and final, visit to drop off their second 24-hour urine sample. At this visit, the subject's weight and blood pressure were taken, and subjects completed an exit survey (Appendix F). A flow chart outlining the study procedures can be found in Appendix G.

## **Laboratory Analyses**

### **24-Hour Urine**

Samples were evaluated from the 24-hour urine collections from the subjects. Urine pH was measured using a Corning® Chek-Mite™ pH tester (Sigma Aldrich, St. Louis, MO). The pH meter was calibrated prior to measurement using a 2-point calibration system with 7.0 and 4.1 pH buffer solutions.



### **Statistical Analyses**

Data obtained was analyzed using SPSS v.22 Statistical Analytical System (SAS, Chicago, IL). All outcome data was checked for normality using the Kolomogrov-Smirov test. All data was normally distributed. Values are described as mean  $\pm$  SD. A one-way ANOVA test was used to compare the mean change in each outcome variable (urinary pH, weight, blood pressure, PRAL, and dietary vitamin C) between the three groups. Data are considered significant when  $p \leq 0.05$ .

## CHAPTER 4

### RESULTS

The purpose of this randomized controlled trial was to determine the effect of consuming a vegan diet 2, 3, and 7-days out of a week on acid-base balance in a healthy college student population. This chapter presents the data collected from the experimental protocol including subject demographics, urinary pH, blood pressure, and weight. Subjects were matched at baseline by gender and BMI to decrease the influence of these variables on the dependent variables. No significant differences in BMI or gender were found between the groups at baseline.

#### **Subject Demographics**

There were 62 responses to the SurveyMonkey survey that was emailed in the listserv advertisement. Out of the 62 respondents, 55 were eligible to participate in the study. A total of 30 volunteers signed a consent form and were enrolled in the trial. The 30 subjects were randomized in blocks of 10. During the randomization process, 3 subjects at a time were matched based on gender and BMI. The 3 subjects were then randomized to 3 different groups by drawing a group number out of a hat. A total of 26 participants completed the study. Four subjects dropped out after enrolling.

Gender, age, weight, height, and BMI were recorded for all of the subjects. A one-way ANOVA test was conducted to determine differences between the 3 groups at baseline. No significant difference were found between the groups in any of the categories as signified by  $p > 0.05$ .

Table 1

*Subject Baseline Demographic & Physical Characteristic Data*

|              | VEG2(n=7) | VEG3(n=10) | VEG7(n=9) | P*   |
|--------------|-----------|------------|-----------|------|
| Gender (M,F) | 2,5       | 2,8        | 1,8       |      |
| Age (years)  | 21.7±1.7  | 22.4±2.6   | 21.4±2.9  | 0.72 |
| Weight(kg)   | 63.6±8.9  | 74.1±30.9  | 64.6±13.3 | 0.55 |
| Height(cm)   | 168.2±3.4 | 165.8±7.4  | 163.9±8.4 | 0.48 |
| BMI          | 22.5±3.1  | 26.5±8.3   | 23.9±3.5  | 0.39 |

\*Data are displayed as mean ±SD. P value represents one-way ANOVA.

**Adherence to Dietary Intervention**

Adherence was determined by assessing the 7-day food logs that were completed during the dietary intervention. The consumption of a non-vegan item on a vegan day resulted in non-adherence for that day. A percent adherence to the dietary intervention was calculated for each subject.

Table 2

*Average % Adherence to Dietary Intervention by Group*

| VEG2 (n=7) | VEG3 (n=7) | VEG7 (n=8) |
|------------|------------|------------|
| 79%        | 95%        | 96%        |

\*Percentages indicate the average percent of days the subjects adhered to their dietary intervention in each group.

### Changes in Outcome Measures

The outcome measures were assessed at baseline and then again upon completion of the dietary intervention. The outcome measures that were assessed were: 24-hour urine pH, systolic blood pressure, diastolic blood pressure, weight, and dietary PRAL. No significant changes were found between the groups in any of the outcome measures ( $p>0.05$ ) except for dietary PRAL ( $p=0.03$ ).

An independent t-test was conducted between gender and the outcome variables to determine whether gender needed to be controlled for. Gender showed no significant impact on any of the outcome variables. Any potential relationships between age, height, and BMI and the outcome variables were assessed through a bivariate correlation statistical test. Again, no relationship was found between these factors and the outcome variables.

A paired t-test was conducted on the baseline urinary pH and the post-test urinary pH in the VEG7 group. A significant difference was observed ( $p=0.05$ ), which indicated that 7 days was adequate time to see a change in urinary pH.

Table 3  
*Differences in Outcome Measures between Groups*

|               |        | VEG2 (n=7) | VEG3(n=8)  | VEG7(n=8)  | P*   |
|---------------|--------|------------|------------|------------|------|
| Urinary pH    | Pre    | 6.4±0.4    | 6.4±0.5    | 6.3±0.4    |      |
|               | Post   | 6.3±0.5    | 6.6±0.5    | 6.8±0.6    |      |
|               | Change | -0.1±0.6   | 0.1±0.5    | 0.5±0.7    | 0.12 |
| Sys BP (mmHg) | Pre    | 110.9±9.1  | 117.9±10.1 | 113.8±15.0 |      |
|               | Post   | 114.4±10.0 | 115.2±11.7 | 113.4±13.0 |      |

|                        |        |                         |                         |                         |      |
|------------------------|--------|-------------------------|-------------------------|-------------------------|------|
|                        | Change | 3.5±6.5                 | -2.8±7.6                | -0.4±8.4                | 0.27 |
| Dia BP (mmHg)          | Pre    | 69.9±6.5                | 75.0±9.8                | 69.1±9.4                |      |
|                        | Post   | 65.6±3.4                | 75.0±9.3                | 67.1±5.6                |      |
|                        | Change | -4.2±4.6                | 0.0±5.3                 | -2.1±5.7                | 0.27 |
| Weight (kg)            | Pre    | 63.6±8.9                | 70.4±28.8               | 63.7±12.7               |      |
|                        | Post   | 64.0±8.9                | 70.5±28.8               | 63.9±12.8               |      |
|                        | Change | .4±0.7                  | .1±0.9                  | .2±1.0                  | 0.84 |
| PRAL**                 | Pre    | 22.7±10.3               | 16.7±10.0               | 29.5±13.2               |      |
|                        | Post   | 6.3±14.6                | 0.1±10.1                | -11.3±14.0              |      |
|                        | Change | -16.4±15.5 <sup>a</sup> | -16.6±16.3 <sup>a</sup> | -42.5±19.4 <sup>b</sup> | 0.03 |
| Dietary vitamin C (mg) | Pre    | 98.2±127.8              | 66.5±67.0               | 58.4±39.4               |      |
|                        | Post   | 105.2±80.5              | 99.3±51.4               | 116.0±50.1              |      |
|                        | Change | 7.0±71.2                | 32.8±67.6               | 73.1±44.3               | 0.24 |

\*Data are displayed as mean ±SD. P value represents one-way ANOVA.

\*\*Potential renal acid load; PRAL (mEq/d) = 0.49 x protein (g/d)+0.037 x phosphorous (mg/d) -0.021 x potassium (mg/d)-0.026 x magnesium (mg/d)-0.013 x calcium (mg/day)

## CHAPTER 5

### DISCUSSION

The purpose of this randomized controlled trial was to determine the effect of consuming a vegan diet 2, 3, and 7-days out of a week on acid-base balance in a healthy college student population. This Chapter presents the discussion including an analysis of the results, limitations of the study, and future considerations.

#### **Analysis of Results**

##### **24-hour urine pH**

The analysis of the data obtained from the 24-hour urine samples of the VEG7 group demonstrated a significant difference between the baseline pH and the post-intervention pH of the urine samples ( $p=0.05$ ). The post intervention urinary pH was significantly higher than at baseline. The VEG2 and VEG3 groups did not see a significant difference in urinary pH from baseline, nor was there a significant difference in urinary pH change between the three groups.

##### **PRAL**

Change in PRAL was significantly different between the three groups ( $p=0.03$ ). Change in PRAL in the VEG7 group was significantly different than the changes in PRAL in VEG2 and VEG3 groups. This indicated that the VEG7 group's dietary acid load decreased more significantly than the VEG2 and VEG3 groups.

#### **Limitations**

As with any dietary intervention study, subject adherence can be difficult to control. For this study, subjects were not given required foods to eat for their dietary intervention. This was done to get an idea of how this dietary pattern would affect a real

population that was consuming this diet, not in a research setting. For this reason, a lot of freedom was given to the volunteers who participated in this study. Because of this, there was a lot of variation in dietary intake, as well as issues with dietary compliance.

As demonstrated by the food logs filled out over the course of the study, caloric intake differed immensely by day during the dietary intervention. It appears as though some subjects did not replace the animal-product-containing foods from their diet, but simply eliminated the. Consequently, they drastically reduced their caloric intake on their vegan days. Because of this, this trial might not have been a good representation of what effect a true vegan diet would have on urinary pH.

Table 4

*Average Caloric Intake throughout the Dietary Intervention by Group*

| Group | Baseline    | Monday      | Tuesday     | Wednesday   | Thurs       | Fri         | Sat         | Sun         |
|-------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| VEG2  | <i>2126</i> | <b>1376</b> | 1789        | 1554        | <b>1581</b> | 1632        | 2499        | 2030        |
| VEG3  | <i>1623</i> | <b>1152</b> | 1779        | <b>1574</b> | 1317        | <b>1350</b> | 1833        | 1576        |
| VEG7  | <i>2023</i> | <b>1565</b> | <b>1614</b> | <b>1537</b> | <b>1459</b> | <b>1453</b> | <b>1434</b> | <b>1513</b> |

\*Bold numbers indicate vegan days

\*\*Italicized numbers indicate average caloric intake at baseline calculated from the 3-day food logs

The food logs also presented more problems. It is very common for subjects to omit food items or underreport quantities that were eaten. It is also possible that had a subject consumed something that was not vegan on their vegan day that they would not report it because they would not want to admit to “cheating” on their dietary intervention. This can cause issues with calculating compliance as well as dietary PRAL.

When adopting a new dietary pattern, it is common to go through a transition period in which one gets used to their new diet and what their go-to foods are. Since this

dietary intervention required people who eat meat daily to go completely vegan for a certain number of days per week, it is difficult to gauge how this dietary pattern would affect health outcomes after one is able to adapt to the new way of eating.

### **Future Considerations**

For a future study, it would be interesting to look at other dietary patterns that could also demonstrate an alkalizing effect. For example, asking subjects to consume a vegan diet every day before 6 pm. Also, it would be a good idea to continue the trial for longer than a week in order for subjects to become more accustomed to the dietary pattern so they don't struggle to make food choices as much, which was an issue for some subjects in this study.

A larger sample size would also be extremely beneficial in a future study. This trial was very much underpowered, which made it very difficult to determine differences between the groups. A larger sample size of about 60 subjects would be ideal to give the study adequate power to demonstrate significant findings.

### **Conclusion**

The present study concluded that following a vegan diet two, three, or seven days out of one week had no significant effect on urinary acidity. No significant between-groups effects were demonstrated for urinary acidity. An increased alkalinity was observed in the urine of the VEG7 group, although this change was not significant. This lack of significance was likely due to the small sample size. The study was significantly underpowered. A sample size of 57 would have been needed to have 80% power. The lack of significant findings is likely due to the small sample size.



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APPENDIX A  
IRB APPROVAL



APPROVAL: EXPEDITED REVIEW

Carol Johnston  
SNHP - Nutrition  
602/827-2265  
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 1/27/2015 the ASU IRB reviewed the following protocol:

|                     |   |
|---------------------|---|
| Type of Review:     | Initial Study   |
| Title:              | Vegan Diets and Health  |
| Investigator:       | Carol Johnston  |
| IRB ID:             | STUDY00002119   |
| Category of review: | (3) Noninvasive biological specimens, (4) Noninvasive procedures, (7)(b) Social science methods, (7)(a) Behavioral research   |
| Funding:            | None  |
| Grant Title:        | None  |
| Grant ID:           | None  |
| Documents Reviewed: | <ul style="list-style-type: none"><li>• Exit survey, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);</li><li>• flyer and verbal script, Category: Recruitment Materials;</li><li>• Health history questionnaire, Category: Screening forms;</li><li>• Survey monkey screener, Category: Screening forms;</li><li>• Vegan diet instructions, Category: Participant materials (specific directions for them);</li><li>• Protocol, Category: IRB Protocol;</li><li>• consent, Category: Consent Form;</li></ul> |



APPENDIX B

INFORMED CONSENT

## **Vegan Diet and Health**

### **INTRODUCTON**

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

### **RESEARCHERS**

Dr. Carol Johnston and Kelly Cosgrove, a Master's student, at Arizona State University Downtown Campus, have requested your participation in a research study.

### **STUDY PURPOSE**

The purpose of the research is to evaluate how differing adherence to a vegan diet impacts health parameters in adults.

### **DESCRIPTION OF RESEARCH STUDY**

You have indicated to us that you are 18-30 years of age, a non-smoker, generally healthy, not a competitive athlete, and eat meat daily. If female, you do not have a history of pregnancy or lactation within the past 6 months. This study will initially involve the completion of brief demographic and health history questionnaires to demonstrate the absence of conditions that may contraindicate health and urine assessments. This research entails that you visit our test facility on three occasions, complete a 7-day food log and two 24-hour urine collections.

The 1<sup>st</sup> lab visit will last about 30 minutes. You will be asked to complete several surveys about your general health. Your height, bodyweight, waist circumference, and blood pressure will be measured. You will be instructed on how to complete a 24 hour urine collection: on two occasions (both on Sundays) you will need to collect all urine for a 24 hour period (starting in the morning and lasting through the following morning); hence, you will need to carry a urine container with you during the day. Participants will be informed of their diet prescription and receive a tutorial for how to adopt a vegan diet. You will also be instructed on how to complete a 7-day diet record. A second visit will be scheduled soon after the 1<sup>st</sup> visit and on a Monday (the day following the first 24-hour urine collection).

The 2<sup>nd</sup> lab visit will be only to drop off the 24 hour urine sample. The 3<sup>rd</sup> lab visit will be scheduled one week after the 1<sup>st</sup> lab visit (the Monday following the 2<sup>nd</sup> urine collection) and last < 30 minutes. You will drop off the 2<sup>nd</sup> urine sample and the 7 day diet record. Your bodyweight, waist circumference, and blood pressure will be measured. You will be asked to complete a short exit survey regarding your perceptions of vegan diets.

Diet prescriptions: participants will be randomized to one of three groups: VEG7 = vegan diet (a vegan diet will be followed for 7 consecutive days); VEG3 = vegan diet 3x / week (a vegan diet day will alternate with a non-vegan diet day for 7 days); or VEG2 = vegan diet 2x / week (a vegan diet day will be followed by 2 non-vegan diet days over the course of 7 days).

**RISKS**

There is little risk for participants in this trial. Adopting a vegan diet for 2, 3, or 7 days over one week is an inconvenience. We will provide a tutorial for planning plant-based meals. Collecting urine for a 24 hour period (on 2 occasions) is inconvenient and will require hand washing after each collection. Urine collection materials will be provided to participants and will permit a greater ease of urine collection. Collections days will be scheduled on Sundays to ease the burden of urine collection. Recording dietary data daily may also be an inconvenience. We will carefully explain these study procedures prior to consenting individuals to be sure they understand what they will be asked to do during the study.

**BENEFITS**

You will not benefit from this study, but you will be provided with all your health marker test results if desired including body fat composition, blood pressure, and acid base balance.

**NEW INFORMATION**

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

**CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. Plasma from blood samples will be stored for 5 years in freezers in the laboratories of the Nutrition Program at Arizona State University after which time they will be disposed of as biohazard waste.

**WITHDRAWAL PRIVILEGE**

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect you any manner.

**COSTS AND PAYMENTS**

You will receive a \$15 Target gift card at the third visit.

**COMPENSATION FOR ILLNESS AND INJURY**

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

**VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 500 N. 3<sup>rd</sup> St., Phoenix, AZ 85004. [602-827-2265]

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

|                     |              |       |
|---------------------|--------------|-------|
| _____               | _____        | _____ |
| Subject's Signature | Printed Name | Date  |

|                      |       |
|----------------------|-------|
| _____                | _____ |
| Contact phone number | Email |

**INVESTIGATOR'S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator \_\_\_\_\_  
Date \_\_\_\_\_

61 APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

## HEALTH HISTORY QUESTIONNAIRE

ID# \_\_\_\_\_

1. Gender: M F

2. Age: \_\_\_\_\_

3. Do you eat meat every day? YES  
NO

4. Are you willing to adhere to a vegan diet for 2, 3, or 7 days over one week? YES  
NO

5. Ethnicity: (please circle one) Native American African-American Caucasian  
Hispanic Asian Other

6. Education (please circle) High school diploma AA/vocational degree College  
degree MS degree PhD degree

7. Do you smoke? No, never \_\_\_\_\_  
Yes \_\_\_\_\_ # Cigarettes per day = \_\_\_\_\_  
I used to, but I quit \_\_\_\_\_ months/years (circle) ago

7. Have you ever been pregnant? YES  
NO

If yes, date of last pregnancy? \_\_\_\_\_

If yes, have you lactated over the past 6 months? YES  
NO

8. Do you take any medications regularly? Yes No *If yes, list type and frequency:*

| <u>Medication</u> | <u>Dosage</u> | <u>Frequency</u> |
|-------------------|---------------|------------------|
|                   |               |                  |
|                   |               |                  |
|                   |               |                  |
|                   |               |                  |

9. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No  
*If yes, list type and frequency:*

| <u>Supplement</u> | <u>Dosage</u> | <u>Frequency</u> |
|-------------------|---------------|------------------|
|                   |               |                  |
|                   |               |                  |
|                   |               |                  |

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10. Have you been hospitalized in the past year ? YES  
NO

If yes, for what? \_\_\_\_\_

11. Please ANSWER (YES/NO) if **you currently have** or if **you have ever** been clinically diagnosed with any of the following diseases or symptoms:

|                        | YES | NO |                        | YES | NO |
|------------------------|-----|----|------------------------|-----|----|
| Coronary Heart Disease |     |    | Chest Pain             |     |    |
| High Blood Pressure    |     |    | Shortness of Breath    |     |    |
| Heart Murmur           |     |    | Heart Palpitations     |     |    |
| Rheumatic Fever        |     |    | Any Heart Problems     |     |    |
| Irregular Heart Beat   |     |    | Coughing of Blood      |     |    |
| Varicose Veins         |     |    | Feeling Faint or Dizzy |     |    |
| Stroke                 |     |    | Lung Disease           |     |    |
| Diabetes               |     |    | Liver Disease          |     |    |
| Low Blood Sugar        |     |    | Kidney Disease         |     |    |
| Bronchial Asthma       |     |    | Thyroid Disease        |     |    |
| Hay Fever              |     |    | Anemia                 |     |    |
| Leg or Ankle Swelling  |     |    | Hormone Imbalances     |     |    |
| Eating Disorder        |     |    | Depression             |     |    |

Please elaborate on any condition listed previously. \_\_\_\_\_

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12. How would you rate your lifestyle?

Not active \_\_\_\_\_ Active \_\_\_\_\_  
Somewhat active \_\_\_\_\_ Very Active \_\_\_\_\_

13. Please circle the total time you spend in each category for an average **week**.

**Light activities** such as:

Slow walking, golf, slow cycling, doubles tennis, easy swimming,  
gardening

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Moderate activities** such as:

Mod. Moderate walking, cycling, singles tennis, moderate swimming,  
weight lifting

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Vigorous activities** such as:

Fast walking/jogging, fast cycling, court sports, fast swimming,  
heavy/intense weight lifting

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

14. Do you consider yourself a competitive athlete? YES

NO

If yes, please explain:

15. How much alcohol do you drink? (average #drinks per day) \_\_\_\_\_

16. Do you have any food allergies? YES

NO

If yes, explain: \_\_\_\_\_

17. Have you ever followed a vegetarian diet? YES

NO

If yes, provide approximate month/year that a vegetarian diet was followed

18. Do you have any other concerns that we should know about if you chose to  
participate in this trial? YES NO

If yes, please elaborate:

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APPENDIX D

VEGAN STARTER HANDOUT

## Label-reading 101

Read food labels in order to avoid hidden animal ingredients. Here are some tips:

- Check **cholesterol** first. Cholesterol is only found in animal foods, so if a food item contains any cholesterol, it is not vegan!
- Next, check the ingredients. Are there any milk, egg, cheese, or milk items? If so, try a different product.
- Make sure there are not any of the common non-vegan ingredients listed below.

Common non-vegan ingredients

- Casein
- Lactose
- Whey

## So what do I eat?

It may seem like a difficult task to eliminate animal foods from your diet, but in reality it's pretty easy! There are many vegan alternatives that are readily-available in most supermarkets that make eating a vegan diet a breeze. Many people find that by switching to a vegan diet they actually eat foods with more variety and flavor than before! There are many recipes and exotic foods you can experiment with while on a vegan diet, but if you are not very savvy in the kitchen, don't worry! There are many simple food choices you can make that require little or no preparation! Here are some simple options of meal ideas that you can adapt to fit your preferences:

## Breakfast

### Option #1

- Oatmeal
- Dried fruit
- Sweetener
- Non-dairy milk (almond, soy, coconut, hazelnut, rice)
- Fruit juice

### Option #2

- Toast
- Nut butter (peanut, cashew, almond)
- Fruit
- Non-dairy milk

### Option #3

- Smoothie
  - Fruits (bananas, berries, kiwi, mango)
  - Non-dairy milk
  - Chia seeds, hemp hearts, sesame seeds

- Greens (kale, spinach)
- Protein (soy, hemp, rice, pea)

## **Lunch**

### Option #1

- Nut butter and jelly sandwich
- Fresh fruit

### Option #2

- Salad
  - Greens (kale, spring mix, romaine lettuce)
  - Fresh veggies (broccoli, bell peppers, tomato, avocado)
  - Seeds/nuts (sunflower seeds, pumpkin seeds, pecans, walnuts)
  - Beans/tofu
  - Dressing
- Granola bar (Clif bar, Lara bar, KIND bar)
- Fresh fruit

### Option #3

- Wrap
  - Tortilla
  - Quinoa or rice
  - Beans
  - Veggies (avocado, tomato, sprouts, bell peppers, onions)
  - Salsa or hummus

## **Dinner**

### Option #1

- Soup/chili
- Toast w/ margarine
- Salad

### Option #2

- Pasta
- Pasta sauce
- Meat alternative (vegan meatballs, texturized vegetable protein)
- Salad

### Option #3

- Stir-fry
  - Veggies (green beans, broccoli, bell peppers, peas, squash, carrots)
  - Tofu, tempeh, meat-alternative
  - Sauce (soy, teriyaki)
- Rice/quinoa

### ***Other resources***

#### Nearby vegan-friendly restaurants

- Green New American Vegetarian
- Loving Hut
- Aside of Heart
- Fair Trade Café
- Zpizza
- Thai Basil
- Pita Jungle
- True Food Kitchen
- Treehouse Bakery
- Nami

#### Vegan blogs (if you want to experiment in the kitchen!)

- Happy Herbivore
- Fat-Free Vegan Kitchen
- Oh She Glows
- Vegan Eats and Treats
- Healthy Happy Life

APPENDIX E

SAMPLE FOOD LOG

### 3-Day Dietary Food Log Instructions

1. Fill in the 3-day dietary food log over the span of 5 consecutive days including 3 week days and 2 weekend days.
2. Please record ALL food and drinks consumed throughout each 24-hour period. Even if it is a handful of chips or a few pieces of candy.
3. Be as specific as you can about the food type and amounts.
4. If the food is a combination food (i.e. a sandwich), include all contents of the meal including any condiments or sauces consumed and the amount of each ingredient.
5. Record the amount in the way that it is ordinarily measured or by using the portion size worksheet attached.
6. Include the brand name when applicable or if the meal is not prepared by you, be as specific as possible with the preparation methods used (i.e. fried, baked, sautéed in oil).
7. Please be honest and be precise.

### Sample Log

| <b>Time</b>     | <b>Food Item</b>     | <b>Amount</b>  | <b>Description</b>                                     |
|-----------------|----------------------|--|--|
| <i>e.g. 9am</i> | <i>e.g. banana</i>   | <i>Give as tsp, tbsp, cups, oz, weight, or portion</i> | <i>Include type of food, brand name, or restaurant</i> |
| 6:30 am         | English Muffin       | 1 Muffin   | Whole wheat, Thomas                                    |
| 6:30 am         | Banana               | 1 Medium Fruit   |  |
| 6:30 am         | Peanut butter        | 2 tbsp   | Low-fat JIFF   |
| 9:30 am         | Yogurt               | 6 oz   | Low-fat Vanilla, Dannon                                |
| 9:30 am         | Granola              | ½ C  | Nature Valley  |
| 12:30 pm        | Spinach              | 3 C  |  |
| 12:30 pm        | Tomato               | ½ C  |  |
| 12:30 pm        | Tofu                 | 3 oz   | Firm   |
| 12:30 pm        | Balsamic Vinaigrette | 2 tbsp   | Kraft  |

### 3-Day Food Log (Day 1)

Date



APPENDIX F  
EXIT SURVEY



Subject \_\_\_\_\_

How important are the following factors when you make food choices?

|                      | Not at all important  | Slightly important    | Very important        |
|----------------------|-----------------------|-----------------------|-----------------------|
| Health               | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Ethics               | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Environmental impact | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Convenience          | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Social influence     | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Taste                | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

How often do you eat the following when consuming your **regular** diet?

|                 | 2-3<br>Times<br>a Day | Once a<br>Day         | 4-6<br>Times<br>a<br>Week | 2-3<br>Times<br>a<br>Week | Once a<br>Week        | 2-3<br>Times<br>a<br>Month | Once a<br>Month       | Never                 |
|-----------------|-----------------------|-----------------------|---------------------------|---------------------------|-----------------------|----------------------------|-----------------------|-----------------------|
| Vegetables      | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Fruits          | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Fruit juice     | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Whole<br>grains | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Red meat        | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Poultry         | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Fish            | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Dairy           | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |
| Eggs            | <input type="radio"/> | <input type="radio"/> | <input type="radio"/>     | <input type="radio"/>     | <input type="radio"/> | <input type="radio"/>      | <input type="radio"/> | <input type="radio"/> |

Which dietary intervention did you follow?

- ☐ Vegan 7 days out of the week
- ☐ Vegan 3 days out of the week
- ☐ Vegan 2 days out of the week

Do you think you could continue following this diet in the future?

- ☐ Yes
- ☐ No
- ☐ Maybe

Explain:

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Did you experience changes in any of the following during the dietary intervention?

|                | Yes                   | Slightly              | Not at all/ Unsure    |
|----------------|-----------------------|-----------------------|-----------------------|
| Energy levels  | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| Digestion      | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |
| General health | <input type="radio"/> | <input type="radio"/> | <input type="radio"/> |

Explain:

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How difficult was it for you to make vegan food choices?

- ☐ Very difficult
- ☐ Difficult
- ☐ Easy
- ☐ Very easy

Explain:

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Please share any other comments about your experience with your dietary intervention:

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APPENDIX G

STUDY PROTOCOL FLOW CHART

ASU Nutrition Listserve

SurveyMonkey

Monday-Wednesday(visit 1):

- Health history/consent
- Provide with 24-hr urine collection materials and instructions
- Plant-based eating tutorial

Thursday-Saturday  
Subjects fill out 3-day food log

Sunday:  
24 hour urine collection

Monday(visit 2):  
Subjects bring 24-hour urine collection to lab

Monday-Sunday:  
Groups undergo their dietary intervention

Sunday:  
24 hour urine collection

Monday(visit 3):

- Subjects bring 24-hour urine collection to lab
- Exit survey